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(54) Title: PEPTIDE COMPOSITIONS AND FORMULATIONS AND USE OF SAME

(57) Abstract

The present invention is directed to a composition which is used to enhance the softness, elasticity, or appearance of tissue. Specifically, the present invention is directed to a composition formulated from peptides which substantially correspond to those produced from thermolysing digestion of elastin. This formulation is preferably applied to human skin in a cosmetic or therapeutic formulation. The present composition specifically includes the known chemical modification of the peptides described herein, specifically carboxy and amino modification including the addition of amino acids to either end of the peptide fragments.

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PEPTIDE COMPOSITIONS AND FORMULATIONS AND USE OF SAME

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to compositions useful as therapeutics or cosmetics. Compositions of the present invention are particularly useful in treating tissue. The compositions of the present invention preferentially include a peptide or peptides which simulate the effect of elastin, and optionally increases the native production of elastin. Preferably, the basic amino acid sequence corresponds to or is homologous with a portion of mammalian elastin, more preferably with fragments of elastin endogenous to the tissue of the mammal being treated. It is preferable that the peptide is at a therapeutically effective concentration and/or is an active ingredient of a pharmaceutic, therapeutic or cosmetic composition. More specifically, the present invention relates to a peptide or plurality of peptides which increase the elasticity and turgor of the skin. Another aspect of the present invention is a method of administering the compositions of the present invention to thereby increase elasticity and/or physical appearance of the skin (*e.g.*, smoother, healthier, and more youthful skin).

2. Background and Description of the Related Art

Skin, in particular mammalian skin, consists of a number of overlapping layers of cells. The outermost layer of mammalian skin is called the stratum corneum. This layer protects mammalian skin from physical and atmospheric harm, acting as a barrier to external dangers. The degree of softness or texture of the stratum corneum is directly dependent on its moisture content. However, it has been found that, in the lower layers of the skin, degenerative changes which occur with age are not caused principally by a lack of moisture. Therefore, even though the texture and appearance of the skin is dependent on the moisture content of the skin, other factors have been shown to influence

the overall appearance and texture of the skin. For example, it has been found that the loss of elasticity in the skin decreases the tone and turgor of the skin. It is speculated that the decrease in skin tone and turgor occurs through degradation of certain complex polypeptides which are present in the skin. These complex polypeptides include elastin and collagen, among others.

Elastin is a highly cross-linked complex polypeptide and is a major component of elastic fibers present in the skin and connective tissue of animals. Elastin appears to be primarily responsible for the physiological elasticity of tissue. In normal mammalian skin, specifically human skin, elastic tissue proteins represent a relatively small fraction of the total dermal proteins, but play a very important role in maintaining or improving the skin tone and structure. Elastin itself is the main protein substance present in elastic fibers and occurs in tendons, blood vessels, and connective tissue. When isolated from these sources, it is normally in the form of a brittle, fibrous, yellowish material which is insoluble in water, alcohol and ether but is somewhat soluble in concentrated aqueous alkali metal hydroxide solutions. The dense cross-linked structure of elastin makes it very difficult to solubilize. There have been many attempts to solubilize elastin and create cosmetic agents from the solubilized elastin. Attempts to solubilize are described for example in a U.S. Patent No. 4,327,078. However, it has been found that elastin is only slightly absorbed by the skin and does not sufficiently penetrate the skin to produce substantial benefits to the skin.

SUMMARY OF THE PRESENT INVENTION

The present invention is directed to compositions which are pharmaceutic, therapeutic, and/or cosmetic to the tissue to which it is applied. The composition of the present invention preferably modifies or appears to modify the physical characteristics of the tissue to which it is applied, and the tissue being modified is preferably mammalian skin tissue. The composition generally includes a vehicle or carrier for therapeutic or cosmetic administration in which the peptides are formulated at therapeutically effective concentrations to increase the elasticity of the skin. The peptides are preferably soluble in

an aqueous solution, and more preferably are comprised of small peptides (usually less than about 10 amino acids in length). It is preferable that the peptide portion of the composition be comprised of peptides having molecular weights of less than 10,000 Da, even more preferably comprised of 90% of peptides having a molecular weight of less than 10,000 Da. Even more preferably the peptide content of the composition is comprised of peptides having a molecular weight of less than about 3,000 Da, even more preferably the peptide content of the composition is comprised of peptides having a molecular weight of less than about 1,000 Da. In fact, it has been found that the preferred molecular weight range of peptides utilized in the present invention is in the range of about 100 - 1,000 Da; more preferred about 150 - 800 Da; even more preferred about 180 - 600 Da; and most preferably the therapeutic or cosmetic composition includes peptides having a molecular weight in the range of about 188 - 585 Da.

It has also been found that the peptides which best accomplish an increase in tissue elasticity and turgor are ones which correspond to or are substantially homologous with portions of elastin, (particularly elastin endogenous to the tissue being treated). Accordingly, it has been found that digestion of elastin, for example hydrolytic or site specific enzymatic cleavage of elastin results in peptides which are particularly suitable for use in the present invention. The peptides which result from digesting can be directly in the pharmaceutic, therapeutic, and/or cosmetic formulation of the present invention. The peptides of the present invention may also be synthesized by those methods known in the art (i.e. solid state, liquid, and over expression). As used herein, the term "peptide" is not meant to convey any meaning regarding the precursor material or methods utilized to synthesize or make the peptides. Additionally, the term "elastin peptide fragment" in either singular or plural form refers to the fact that the peptide or amino acid sequence being discussed corresponds to, is the biological equivalent of, or is substantially homologous with, a portion of elastin, more specifically to a portion or fragment of elastin endogenous to the animal being treated. However, the term "elastin peptide fragment" is not meant to convey any meaning regarding the source or starting material or method of arriving at the elastin peptide fragment. As stated above, peptides

of the present invention are preferably formed by enzymatic cleavage of elastin and are even more preferably formed by cleavage of elastin with thermolysin to form hydrophilic elastin derived peptides. It is also preferable that the peptides of the present invention are at an effective concentration within the therapeutic or cosmetic composition, wherein the therapeutically effective concentration is in a range of about .0002% to about 90% by weight of peptide, more preferably in a range of about .05% to about 50% peptide, even more preferably in a range of about 0.5% to about 10% peptide, even more preferably about 1.5% peptide, and most preferably about 1.3% hydrolyzed elastin peptide. The therapeutic composition of the present invention can be formulated as a cosmetic preparation to be applied topically to a patient's skin, such as in an emulsion, lotion, spray, powder, ointment, cream, or foam or in other suitable pharmaceutical vehicles or carriers commonly known in the art for other types of administration (i.e., subcutaneous). The delivery system of the present invention is preferably a topical delivery system but also may be a subcutaneous, transcutaneous, oral, nasal, aerosol, or patch delivery system.

The present invention is further directed to a composition for improving tissue texture, wherein the composition is comprised of an elastin peptide which formed by selective cleavage of elastin. Preferably the composition includes a pharmaceutical delivery system and the elastin is derived from animal tissue. *Ligamentum nuchea* has been found to be a particularly useful source of elastin starting material. The elastin of the present invention is preferably selectively cleaved by enzymatic digestion of the elastin with thermolysin. This thermolytic cleavage preferably results in a elastin peptide fragment or fragments having a molecular weight of less than about 10,000 Da, more preferably less than about 3,000 Da, even more preferably less than about 1,000 Da.

The present invention is also directed to a method of enhancing the functionality, tone, turgor, and/or elasticity of the tissue to which it is administered by administrating effective amounts of a peptide to the tissue (particularity skin tissue). When treating skin, the appearance of the skin is enhanced, it is believed as a consequence of improving the elasticity of the tissue to which the peptide is applied. It is preferable that the administration step be comprised of a number of separate

administration steps which are repeated most preferably twice daily over a predetermined time, wherein the predetermined time exceeds one week of daily administration of the peptide, more preferably two weeks, and most preferably at least a month of daily topical application (with twice daily of the peptide administration over the month being even more preferable.). As with the composition of the present invention, in the method of the present invention, it is preferable that the composition utilized include an elastin peptide fragment comprised of peptides having a molecular weight of less than about 1,000 Da. Preferably and conveniently, the peptide can be formed by enzymatic cleavage of the elastin (preferably derived from animal tissue). It is preferable that the enzymatic cleavage occurs via enzymatic treatment of purified elastin starting material with thermolysin.

BRIEF DESCRIPTION OF THE DRAWINGS

The features, aspects, and advantages of the present invention will become better understood in light of the following description, appended claims, and accompanying drawings wherein:

Fig. 1 is a bar graph illustrating increased elastin production as a result of application of the present invention to mammalian skin.

Fig. 2 is a micrograph illustrating the microvascular response of the skin tissue with the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In order that the invention herein described may be more fully understood, the following detailed description is set forth. The present invention relates to compositions which are useful in increasing elasticity, turgor, and/or appearance of tissue.

The present invention is also directed to administering therapeutically effective concentrations of the compositions.

As used herein, the term "subject" or "patient" means any mammal, including humans, in which elastin is utilized for proper tissue function or appearance. The methods herein for use contemplate prophylactic, cosmetic, and curative use.

As used herein, the term "about" means plus or minus 10% of the numerical value of the number with which it is being used. Therefore, about 50% means in the range of 45%-55%. As used herein, the term "Dalton" (or "Da") refers to the unit of mass which is equivalent to the mass of a hydrogen atom (1.66×10^{-24} gram). Generally speaking, the term "tissue" refers to any aggregation of similarly specialized cells which are united in the performance of a particular function. As used herein, "tissue", unless otherwise indicated, refers to tissue which includes elastin as part of its necessary structure and/or function. For example, connective tissue which is made up of, among other things, collagen fibrils and elastin fibrils satisfies the definition of "tissue" as used herein. Additionally, elastin appears to be involved in the proper function of blood vessels, veins, and arteries in their inherent visco-elasticity. Unless otherwise indicated, the term "skin" means that outer integument or covering of the body, including the dermis and the epidermis and resting upon subcutaneous tissue.

"Providing" when used in conjunction with a therapeutic means to administer a therapeutic directly into or onto a target tissue or to administer a therapeutic to a patient whereby the therapeutic positively impacts the tissue to which it is targeted (either in a prophylactic, curative or cosmetic manner. Thus, as used herein, the term "providing", when used in conjunction with elastin peptide fragment, can include, but is not limited to, providing an elastin peptide fragment into or onto the target tissue; providing an elastin peptide fragment systemically to a patient by, e.g., intravenous injection whereby the therapeutic reaches the target tissue; providing an elastin peptide fragment in the form of the encoding sequence thereof to the target tissue (e.g., by so-

called gene-therapy techniques) whereby the elastin peptide fragment is expressed within the target tissue.

Details on techniques for formulation and administration of pharmaceuticals may be found in the latest edition of Remington's Pharmaceutical Sciences (Mack Publishing Co, Easton Pa.). Although local topical delivery is desirable, there are other means of delivery, for example: oral, parenteral, aerosol, intramuscular, subcutaneous, transcutaneous, intamedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration.

As used herein, the term "therapeutic" means an agent utilized to treat, combat, ameliorate, prevent or improve a condition or disease of a patient. The condition being treated in the present invention is deficient elastin in tissue, that is, a need in the tissue for more elastin. As it applies to skin, it is measured by turgor, tone, appearance, degree of wrinkles, and youthfulness. As the term applies to blood vessels it may be measured by the degree of elasticity or proper vasomotor response (vasodilatation/vasoconstriction) of the vessel. Accordingly, therapeutic treatment of blood vessels may have implications in diseases associated with visco-elasticity, including hypertension, arteriosclerosis, angina, angiogenesis, myocardial infarction, coronary thrombosis, restenosis post angioplasty, and chronic obstructive pulmonary disease.

Finally, the term "cosmetic," as used herein, refers to a beautifying substance or preparation which preserves, restores, bestows, simulates, or enhances the appearance of bodily beauty, specifically as it relates to the appearance of tissue or skin.

As stated above, the present invention is directed to an elastin peptide fragment which is useful as a therapeutic and or cosmetic composition or agent for modifying tissue, especially skin. The term "modify" is used to convey that the present invention changes either the appearance, form, characteristics and/or the physical attributes of the tissue to which it is being provided, applied or administered. The change

in form can be reflected in any of the following alone or in combination: enhanced appearance of the skin; increased softness of the skin; increased turgor of the skin; increased texture of the skin; increased elasticity of the skin; decreased wrinkle formation and increased endogenous elastin production in the skin.

The source of the starting elastin material can derive from a number of sources known in the art. It is known, for example, that the *ligamentum nuchae* is made up largely of elastin, with only a relatively small amount of collagen. More than 70% of the dry weight of this ligament is elastin. Due to the relatively high elastin content and relatively low collagen content, *ligamentum nuchae* is an ideal starting material to use in deriving the elastin peptide fragments of the present invention. The *ligamentum nuchae* may be cleaned first using a procedure similar to that disclosed in U.S. Patent No. 5,028,695, this portion of which is incorporated herein by reference thereto. Although the preferred source of the starting material of the present invention is *ligamentum nuchae*, other ligaments, tendons, connective tissue, tissue, and synthetic sources may also be used. For example, the arteries and lungs, and other animal tissue, especially those which have significant amounts of elastin, can be used. Also, elastin from different sources, or elastin and collagen from the same or different sources could be mixed together to produce a particular advantageous mix suitable for an intended use. For example, rat, sheep, and porcine aorta can be used as a source of elastin as described in L.B. Sandberg, *Connective Tissue Research*, 1990, Vol. 25, pp. 139-148, which is hereby incorporated herein in its entirety by reference thereto.

In the present invention, the ligament extraction is comprised of taking dissected *ligamentum nuchae* ligaments and removing as much fat and excess connective tissue as possible. These "clean" ligaments are then chopped into about one centimeter square (1 cm^2) pieces and washed with doubly distilled water ("DDW"). The clean ligaments are then placed on a metal mortar, pre-chilled to -20°F and liquid nitrogen is added to freeze the tissue. The ligaments are then minced or pulverized with the appropriate tool and re-suspended in 1% aqueous NaCl at a ratio of about 100 grams of tissue to about three liters of 1% aqueous NaCl and homogenized in a Waring blender at

high speed for 30-60 seconds. The homogenized ligament is transferred to a four-liter beaker and stirred overnight at 4°C on a magnetic stirrer, after which it is centrifuged at 32,500 x G and the supernatant is checked for protein content using the Biuret method for protein determination. The Biuret reaction is done by mixing 2 milliliters of extract with 3 milliliters of reagent and reading immediately either by simple visual inspection or at 540 nanometers on a spectrophotometer to determine the protein concentration of the supernatant. The supernatant is then discarded. The pellet (referred to hereinafter as the elastin pellet) is resuspended in 1% aqueous NaCl and homogenized. The process of homogenizing in a Waring blender, stirring overnight and centrifuging are repeated three to four times until the supernatant is Biuret negative. After centrifugation, the elastin pellet is resuspended in DDW and autoclaved 30 psi for six hours. The resuspended elastin pellet is centrifuged again and the supernatant is tested for protein content via the Biuret method. The elastin is washed with boiling DDW and then with DDW at room temperature and the washes are tested for protein content via the Biuret method. If the washes are Biuret negative, the elastin pellet is dried with chloroform/methanol solution at a ratio of 2 parts chloroform to 1 part methanol. If the Biuret test is positive, the six hour autoclave procedure with wash step is repeated until the Biuret test is negative. Finally, the elastin residue is washed with five volumes of pure methanol and air-dried at room temperature. The elastin residue is transferred to a desiccator and dried over P_2O_5 for 24 hours until the weight of the elastin residue is stable. The elastin residue is then milled in a Willey mill through a 40-mesh screen followed by a 60-mesh screen.

For the thermolysin digestion, three times re-crystallized thermolysin product from CalBiochem (10394 Pacific Center Court, San Diego, CA 92121) was used. The thermolysin preparation contains sufficient calcium to ensure maximal activity of the enzyme. The thermolysin digestion is done as follows: a waterbath is brought to a 55° C temperature with a rotary shaker and five grams of the finely milled largely insoluble elastin residue is hydrated with one liter of DDW for fifteen minutes at room temperature. After hydration, the one liter DDW which contains the five grams of elastin is placed in the 55° C bath and the pH of the elastin/water mixture is brought to a pH between 7-8

with 10% methylamine. Fifty milligrams of thermolysin (*bacillus thermoproteolyticus*) is added directly to the elastin water mixture. The thermolysin contains about 60% protein (60.2%), about 13% (13.2%) sodium acetate, and about 25% (25.3%) calcium acetate, with a specific activity of about 8,720 I.U./mg dry weight. The pH of the elastin water mixture is monitored with a pH meter or pH stat and adjusted with 10% methylamine to keep the pH between 6.8 and 7.5. The digestion is allowed to continue for 75 minutes and then concentrated hydrochloric acid is added to adjust the pH to 3.0 to terminate the digestion.

After digestion is terminated, the digested product is preferably filtered through a PM 10 Diaflow 10,000 molecular weight cut-off ultra-filtration membrane to filter out any protein or peptides exceeding about 10,000 Da molecular weight. The resulting supernatant is a derived composition comprised of peptides having a molecular weight of less than about 10,000 Da. As it turns out, the most preferred composition is comprised of an elastin peptide fragment with a molecular weight of less than about 1,000 Da. Table 1 is a list of peptide sequences isolated from the thermolytic cleavage of elastin. These isolated fractions, either alone or in combination, when applied to tissue, result in the tissue, specifically mammalian skin, exhibiting characteristics of increased skin elasticity, including skin softness and increased turgor as well as an overall increase in the attractiveness of the skin. As can be seen from Table 1 below, it is preferable that the composition of the present invention be comprised of elastin peptide fragments having an amino acid chain length of less than about 10 amino acids or having a molecular weight in the range of about 150 - 800 Da, even more preferably about 180 Da to about 600 Da, and most preferably from about 188 Da to about 585 Da. It is also preferable that the peptide or peptides used in formulating the composition of the present invention are comprised substantially of amino acids having an apolar and/or an uncharged side group (i.e. alanine, valine, proline, glycine), more preferably comprised of peptides which include valine or proline, and even more preferably comprised of peptides containing valine and proline in each amino acid sequence.

The elastin peptide fragments which have been identified in the present invention have the following amino acid sequences:

SEQ #	PEPTIDE	MOL WT	NAME (N- to C-terminal)	c-DNA Copies
1.	AVG	245	Alanine-Valine-Glycine	1
2.	VGAG	302	Valine-Glycine-Alanine-Glycine	2
3.	IGG	302	Isoleucine-Glycine-Glycine	4
4.	LG	188	Leucine-Glycine	26
5.	IGAG	316	Isoleucine-Glycine-Alanine-Glycine	2
6.	LGG	245	Leucine-Glycine-Glycine	6
7.	VAPG	342	Valine-Alanine-Proline-Glycine	2
8.	LGPG	342	Leucine-Glycine-Proline-Glycine	3
9.	LGAG	316	Leucine-Glycine-Alanine-Glycine	4
10.	VGPG	328	Valine-Glycine-Proline-Glycine	2
11.	FGPG	376	Phenylalanine-Glycine-Proline-Glycine	2
12.	VGPQ	399	Valine-Glycine-Proline-Glutamine	1
13.	LGA	259	Leucine-Glycine-Alanine	7
14.	VGPA	342	Valine-Glycine-Proline-Alanine	1
15.	VVPG	370	Valine-Valine-Proline-Glycine	2
16.	AVPG	342	Alanine-Valine-Proline-Glycine	2
17.	VVPQ	441	Valine-Valine-Proline-Glutamine	1
18.	VAARPG	569	Valine-Alanine-Alanine-Arginine-Proline-Glycine	1
19.	LGAGGAG	501	Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine	1
20.	AIPG	356	Alanine-Isoleucine-Proline-Glycine	2
21.	LGPGG	399	Leucine-Glycine-Proline-Glycine-Glycine	1
22.	AAAQA	430	Alanine-Alanine-Alanine-Glutamine-Alanine	1
23.	VGVHypG	444	Valine-Glycine-Valine-Hydroxyproline-Glycine	14*
24.	VYPGG	491	Valine-Tyrosine-Proline-Glycine-Glycine	1
25.	IGGVGG	458	Isoleucine-Glycine-Glycine-Valine-Glycine-Glycine	1

SEQ #	PEPTIDE	MOL WT	NAME (N- to C-terminal)	c-DNA Copies
26.	VAPGVG	498	Valine-Alanine-Proline-Glycine-Valine-Glycine	1
27.	LGVGG	401	Leucine-Glycine-Valine-Glycine-Glycine	3
28	VLPG	384	Valine-Leucine-Proline-Glycine	3
29.	FRAAA	534	Phenylalanine-Arginine-Alanine-Alanine-Alanine	1
30.	VGGVPG	484	Valine-Glycine-Glycine-Valine-Proline-Glycine	1
31.	FGPGG	433	Phenylalanine-Glycine-Proline-Glycine-Glycine	1
32.	VGVPG	427	Valine-Glycine-Valine-Proline-Glycine	14*
33.	VLPGAG	512	Valine-Leucine-Proline-Glycine-Alanine-Glycine	1
34.	VGLHypG	458	Valine-Glycine-Leucine-Hydroxyproline-Glycine	1**
35.	LGVGA	415	Leucine-Glycine-Valine-Glycine-Alanine	1
36.	AFPG	390	Alanine-Phenylalanine-Proline-Glycine	1
37.	AFPGA	461	Alanine-Phenylalanine-Proline-Glycine-Alanine	1
38.	VGIPA	455	Valine-Glycine-Isoleucine-Proline-Alanine	1
39.	VGGIPT	542	Valine-Glycine-Glycine-Isoleucine-Proline-Threonine	no
40.	VGVGVPG	583	Valine-Glycine-Valine-Glycine-Valine-Proline-Glycine	2
41.	LGPGVG	498	Leucine-Glycine-Proline-Glycine-Valine-Glycine	1

* Sequence Nos. 23 and 32 appear to be a common sequence because Proline hydroxylation is a post-translational event.

** as VGLPG.

The above sequences account for about 40% of all the elastin sequences with the rest of the sequences being reduced to free amino acids or desmosine crosslinks (these amino acids are not being accounted for with sequencing).

The elastin peptide fragment/water mixture which is obtained upon digestion with thermolysin described is preferably flash evaporated to dryness and

redissolved in a small volume of DDW and if desired is diluted sufficiently with DDW for lyophilization to dryness. In the alternative, rather than redissolving the elastin peptide(s), the filtered product is freeze dried twice, resulting in a powder which contains 30 weight chemically-bound water and very little salt (NaCl). Preferably the powder for therapeutic use is dissolved to a concentration of about .0002% to about 90% by weight of elastin peptide fragment, more preferably in a range of about .05% to about 50% , even more preferably in a range of about .05% to about 10% elastin peptide fragment, and more preferably about 1.5% elastin peptide fragment, and most preferably about 1.3% peptide fragment or fragments in a vehicle which is suitable for topical or subcutaneous administration.

As can be seen from Fig. 1, the topical treatment with a composition which included peptide fragments at a concentration of about 1.3% when applied to the skin of a Sprague-Dawley male rat over a one month period illustrates a doubling of the elastin content of the skin, as compared to both control samples and similar applications and concentration of DHEA. In Figure 1, S CONTR represents the Shaven Control and US CONTR represents the Unshaven Control. Figure 1 illustrates that the present invention has the advantageous qualities of enhancing the softness or elasticity of the skin by increasing the endogenous production of elastin in the skin. The peptides and formulations of the present invention also appear to improve the texture of skin, specifically the physical appearance of the skin by improving the endogenous production of elastin.

The method of administering peptides and formulations of the present invention employs any of a number of known administrative routes such as oral, IV, subcutaneous, transcutaneous, and topical administration. A preferred method of the present invention employs a pharmaceutical or cosmetic composition which enhances the physical appearance of and/or the elasticity of tissue. It is believed that the limit for skin penetration of elastin peptide fragment is a molecular weight of about 20,000 Da. Advantageously, the present invention uses peptides derived from elastin through thermolytic cleavage which have a molecular weight of less than about 10,000 Da, more

preferably less than about 3,000 Da, even more preferably less than 1,000 Da. Thus, the peptides of the present invention would appear to meet the criteria ****** be absorbed by the skin upon application. Beyond the increased absorption due to the relative small size of the active peptides of the present invention, the peptides themselves which preferably correspond to those formed through thermolytic cleavage of elastin with thermolysin, appear to have increased activity. It is thought that this activity is at least in part due to the production of endogenous elastin on skin to which the administration or the composition is applied.

The present invention can be formulated in a number of carrier vehicles, for example, in a spray; an aerosol; a water and an oil-type emulsion; an oil and water-type emulsion; a face cream or body cream; a sun lotion or after-sun lotion; or other topical administration vehicle. U.S. Patent No. 4,327,078, which was referenced earlier, is illustrative of the different types of topical administrations which may be employed to administer a soluble elastin-based derivative. In each of the examples provided, the concentration of the elastin peptide fragment of the present invention would be preferably about 1.5% and the concentration of water would be increased to make up the difference.

It is preferable that the topical administration of the composition of the present invention occur repeatedly over a predetermined time period, preferably in the range of about one week to about one month. In the Sprague-Dawley rats used to generate Fig. 1, the rats were treated topically with a 1.3% concentration of the hydrophilic elastin peptide formulated by the method disclosed herein for a period of 30 days. Testing illustrated that the endogenous elastin (measured by microgram (μg) Elastin per milligram (mg) Skin Fat Free Dry weight) of each of the rats to which the administration was applied doubled over that of a control sample and to a sample which was treated with a 5% concentration of DHEA over a similar time period. Three animals each were used to generate the data for S CONTR, US CONT, and DHEA and eleven animals were used for HEP. Three skin samples from the treated areas of each animal were taken for study, and the three results from each animal were averaged. The mean of these results were: S CONTR (1.408); US CONTR (2.291); DHEA (1.753); HEP

(3.175). The elastin content of the skin was determined by a precise assay for rat elastin developed by Sandberg, et al. ("Quantitation of Elastin in Tissues and Culture: problems related to the accurate measurement of small amounts of elastin with special emphasis on the rat" *Connective Tissue Research.* 25: 139-48, 1990) the assay portion of which is hereby incorporated herein by reference thereto. An alpha level less than 0.001 for the data of Fig. 1 as determined by analysis of variance, is significant because there is less than one chance in a thousand that the findings occur by chance.

The data of Fig. 1 further supports the use of the cosmetic or pharmaceutical preparation over an extended period preferably in the range of one week to one month, more preferably in the range of seven days to about fourteen days and most preferably about fourteen days of daily administration at about 1.5% concentration of elastin peptide or peptides having a molecular weight lower than about 10,000 Da, more preferably less than 1,000 Da and most preferably in the range of about 180 Da to about 600 Da.

Figs. 2A-2D are micrographs illustrating an increased appearance and beneficial cosmetic implication of the present invention. From Figs. 2A-2D, skin treated with an elastin peptide fragment appears to be healthier than untreated skin. This is evidenced under a microscope by an increase in vascular response. In Figs. 2A-2D, fixed tissue sections of rat skin were labeled with fluorescein conjugated antifibronectin antibodies. Fig. 2A is a representative sample from the unshaven control tissue; Fig. 2B is a representative sample from the shaven control sample; and Fig. 2C is a representative sample of the tissue which received DHEA topical treatment. Finally, Fig. 2D received treatment with the present invention in a topical treatment in accordance with the samples discussed above with regard to Fig. 1. The dermal layer in the control panels (Figs. 2A and 2B) is relatively uniform and thin compared to the thickness of both Fig. 2C and Fig. 2D. For convenience, in each of Figs. 2A, 2B, 2C and 2D, the dermal layer is bracketed. Surprisingly and illustrative of some of the benefits obtained utilizing the present invention, Fig. 2D illustrates an increased concentration of capillary venules in the

subdermal region. The capillary venules are shown in this figure as brightly stained oval bodies that lie beneath the dermal layer. The increase in the concentration of endothelial cells in the subdermal region indicate an increase in capillary density and therefore illustrate the potential for the peptides and formulations of the present invention to be used for the formation of blood vessels or capillary venules (neovascularization or angiogenesis).

It appears that the elastin peptide fragment of the present invention would preferably include sequences of Valine-Valine-Proline-Glutamine or Valine-Glycine-Valine-HydroxyProline (possibly Proline)-Glycine. It would also appear that sequences which contain Valine and/or Proline are also preferred, and that peptides which include either or both of these amino acids in a larger concentration (relative to other amino acids present) are most preferred.

As can be seen from Table II below, it would appear that certain groups of the peptides described herein (*e.g.*, Sequence No. 1-Sequence No. 41 inclusive) have preferred characteristics as they relate to cosmetic or therapeutic application to the skin. The elastin peptide mixture isolated from thermolysin digestion of elastin (*i.e.*, Sequence No. 1-Sequence No. 41 inclusive) was collected as they came off of a HPLC column. Instead of isolating each of the thermolysin peptide fragments individually, 5 fractions or cluster of peptides were collected in the 5-50 minute range and were tested for activity utilizing a bromodeoxyuridine Triphosphate (BrdUTP) incorporation assay. The assay measures production of mRNA involved in protein synthesis. Table II measures the green fluorescence intensity as a measure of increased mRNA in RFL-6 cells in response to the pooled elastin fragment.

Table II

Fraction #	Approximate Elution time	Approximate % Change w/Control Subtracted Out
1	5.3 min. - 11.8 min	1%
2	11.8 min - 23.0 min	4%
3	23.0 min - 44.1 min	41%
4	44.1 min - 45.8 min	10%
5	45.8 min - 50.0 min	2%
6	unfractionalized mixture (Seq. No. 1-41)	52%

Each of the fractions show an increase in mRNA in RFL-6 cells over the control group. From the test, however, it appears that Fraction #3 alone and/or in combination with other fractions (*e.g.*, as seen with Fraction #6) has good potential as a composition or formulation to increase elasticity, turgor, and/or appearance of tissue, specifically skin. Fraction 3 includes Sequence Nos. 14-31. It should be noted that in light of the case in obtaining the unfractionalized mixture (as described above) it may be more preferable to use the unfractionalized mixture than isolating the most active ingredient.

Fraction or Cluster 3 was sub-fractionated into 10 fractions corresponding to the ten major peaks identified on the HPLC (at 215 nm). Table III below illustrates the green fluorescence intensity as a measure of increased mRNA in RFL-6 cells in response to sub-fractionated portions of Fraction No. 3 shown in Table II above.

Table III

Fractionated #	Patent Seq. No. Contained Therein	Abbreviated Peptide Sequence	% Change of Green Fluorescence Intensity
1	Sequence No. 14	VGPA	39
2	Sequence No. 15, 16	VVPG, AVPG	40
3	Sequence No. 17	VVPQ	85
4	Sequence Nos. 18, 19	VAARPG, LGAGGAG	44
5	Sequence Nos. 20, 21	AIPG, LGPGG	42
6	Sequence No. 22	AAAQA	20
7	Sequence No. 23	VGVHypG	57
8	Sequence No. 24	VYPGG	38
9	Sequence Nos. 25, 26, 27, 28, 29	IGGVGG, VAPGVG, LGVGG, VLPG, FRAAA	10
10	Sequence Nos. 30, 31	VGGVPG, FGPGG	23
Blank			30

As can be clearly seen from Table III, Sequence No. 17 (VVPQ) has the greatest activity, followed by Sequence No. 23 (VGVHypG) and then Sequence Nos. 18 (VAARPG) and 19 (LGAGGAG). It would appear that Sequence Nos. 22 and 25-31 actually adversely impact the overall therapeutic or cosmetic value of Fraction 3. However, applicant does not wish to be bound by this speculation in that any one or combination of these fractions while lowering the green fluorescence intensity of the fractionated sample may in fact add a desirable characteristic to the intended use of the overall mixture or when combined with another peptide (*e.g.*, any of Seq. No. 1-41 respectively).

While the foregoing has been set forth in considerable detail, the sequences are presented for elucidation, and not limitation. Modifications and improvements, including equivalents, of the technology disclosed above which are within the purview and abilities of those in the art are included within the scope of the claims

appended hereto. It will be readily apparent to those skilled in the art that numerous modifications, alterations and changes can be made with respect to the specifics of the above description without departing from the inventive concept described herein. For example, the peptides and formulations can be administered via many alternative drug delivery vehicles known in the art and the peptides can be derived from digestion of elastin or by amino acid sequencing (either solid state or liquid), as well as from over-expression in a bacterial system. Modification (either chemical or enzymatic) of the basic sequences described herein are also within the purview of the present invention. For example, it appears that a reoccurring pattern in the elastin sequence is the presence of a glycine-alanine residue. Therefore the Seq. No. 1-41 may be modified to include this residue at either the amino or carboxy ends of the peptides. The sequences may also be chemically modified to increase their activity (*e.g.*, amidation of the carboxy terminus portion of a sequence). Accordingly, all such variances should be viewed as being within the scope of the present invention as set forth in the claims below.

WHAT IS CLAIMED IS:

1. A composition useful in treating a condition of mammalian tissue, said composition being comprised of an elastin peptide fragment or a biological equivalent.

2. The composition of claim 1, wherein said elastin peptide fragment is soluble in an aqueous solution.

3. The composition of claim 1, wherein said elastin peptide fragment is at a therapeutically effective concentration.

4. The composition of claim 1, wherein said elastin peptide fragment is selected from the group consisting of Sequence No. 1 (Alanine-Valine-Glycine), Sequence No. 2 (Valine-Glycine-Alanine-Glycine), Sequence No. 3 (Isoleucine-Glycine-Glycine), Sequence No. 4 (Leucine-Glycine), Sequence No. 5 (Isoleucine-Glycine-Alanine-Glycine), Sequence No. 6 (Leucine-Glycine-Glycine), Sequence No. 7 (Valine-Alanine-Proline-Glycine), Sequence No. 8 (Leucine-Glycine-Proline-Glycine), Sequence No. 9 (Leucine-Glycine-Alanine-Glycine), Sequence No. 10 (Valine-Glycine-Proline-Glycine), Sequence No. 11 (Phenylalanine-Glycine-Proline-Glycine), Sequence No. 12 (Valine-Glycine-Proline-Glutamine), Sequence No. 13, (Leucine-Glycine-Alanine), Sequence No. 14 (Valine-Glycine-Proline-Alanine), Sequence No. 15 (Valine-Valine-Proline-Glycine), Sequence No. 16, (Alanine-Valine-Proline-Glycine), Sequence No. 17 (Valine-Valine-Proline-Glutamine), Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine), Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine), Sequence No. 20 (Alanine-Isoleucine-Proline-Glycine), Sequence No. 21 (Leucine-Glycine-

Proline-Glycine-Glycine), Sequence No. 22 (Alanine-Alanine-Alanine-Glutamine-Alanine), Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine), Sequence No. 24 (Valine-Tyrosine-Proline-Glycine-Glycine), Sequence No. 25 (Isoleucine-Glycine-Glycine-Valine-Glycine-Glycine), Sequence No. 26 (Valine-Alanine-Proline-Glycine-Valine-Glycine), Sequence No. 27 (Leucine-Glycine-Valine-Glycine-Glycine), Sequence No. 28 (Valine-Leucine-Proline-Glycine), Sequence No. 29 (Phenylalanine-Arginine-Alanine-Alanine-Alanine), Sequence No. 30 (Valine-Glycine-Glycine-Valine-Proline-Glycine), Sequence No. 31 (Phenylalanine-Glycine-Proline-Glycine-Glycine), Sequence No. 32 (Valine-Glycine-Valine-Proline-Glycine), Sequence No. 33 (Valine-Leucine-Proline-Glycine-Alanine-Glycine), Sequence No. 34 (Valine-Glycine-Leucine-Hydroxyproline-Glycine), Sequence No. 35 (Leucine-Glycine-Valine-Glycine-Alanine), Sequence No. 36 (Alanine-Phenylalanine-Proline-Glycine), Sequence No. 37 (Alanine-Phenylalanine-Proline-Glycine-Alanine), Sequence No. 38 (Valine-Glycine-Isoleucine-Proline-Alanine), Sequence No. 39 (Valine-Glycine-Glycine-Isoleucine-Proline-Threonine), Sequence No. 40 (Valine-Glycine-Valine-Glycine-Valine-Proline-Glycine), and Sequence No. 41 (Leucine-Glycine-Proline-Glycine-Valine-Glycine).

5. The composition of claim 1, wherein said elastin peptide fragment is selected from the group consisting of Sequence No. 14 (Valine-Glycine-Proline-Alanine), Sequence No. 15 (Valine-Valine-Proline-Glycine), Sequence No. 16, (Alanine-Valine-Proline-Glycine), Sequence No. 17 (Valine-Valine-Proline-Glutamine), Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine), Sequence No. 19 (Leucine-Glycine-

Alanine-Glycine-Glycine-Alanine-Glycine), Sequence No. 20 (Alanine-Isoleucine-Proline-Glycine), Sequence No. 21 (Leucine-Glycine-Proline-Glycine-Glycine), Sequence No. 22 (Alanine-Alanine-Alanine-Glutamine-Alanine), Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine), Sequence No. 24 (Valine-Tyrosine-Proline-Glycine-Glycine), Sequence No. 25 (Isoleucine-Glycine-Glycine-Valine-Glycine-Glycine), Sequence No. 26 (Valine-Alanine-Proline-Glycine-Valine-Glycine), Sequence No. 27 (Leucine-Glycine-Valine-Glycine-Glycine), Sequence No. 28 (Valine-Leucine-Proline-Glycine), Sequence No. 29 (Phenylalanine-Arginine-Alanine-Alanine-Alanine), Sequence No. 30 (Valine-Glycine-Glycine-Valine-Proline-Glycine), and Sequence No. 31 (Phenylalanine-Glycine-Proline-Glycine-Glycine).

6. The composition of claim 1, wherein said elastin peptide fragment is selected from the group consisting of Sequence No. 14 (Valine-Glycine-Proline-Alanine), Sequence No. 15 (Valine-Valine-Proline-Glycine), Sequence No. 16, (Alanine-Valine-Proline-Glycine), Sequence No. 17 (Valine-Valine-Proline-Glutamine), Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine), Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine), Sequence No. 20 (Alanine-Isoleucine-Proline-Glycine), Sequence No. 21 (Leucine-Glycine-Proline-Glycine-Glycine), Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine), and Sequence No. 24 (Valine-Tyrosine-Proline-Glycine-Glycine).

7. The composition of claim 1, wherein said elastin peptide fragment is Sequence No. 17 (Valine-Valine-Proline-Glutamine).

8. The composition of claim 1, wherein said elastin peptide fragment is Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine).

9. The composition of claim 1, wherein said elastin peptide fragment is Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine).

10. The composition of claim 1, wherein said elastin peptide fragment is Sequence No. 32 (Valine-Glycine-Valine-Proline-Glycine.

11. The composition of claim 1, wherein said elastin peptide fragment has a molecular weight of less than about 1,000 Da.

12. The composition of claim 1, wherein said elastin peptide fragment is formed by digestion of elastin with thermolysin.

13. The composition of claim 3, wherein said therapeutically effective concentration is a range of about .0002% to about 90%.

14. The composition of claim 1, wherein said therapeutically effective concentration is in the range of about 0.5% to about 10%.

15. The composition of claim 1, wherein said composition is a cosmetic preparation.

16. The composition of claim 15, wherein said cosmetic preparation is formulated as a topical preparation to be applied to a patient's skin.

17. The composition of claim 16, wherein said topical preparation is selected from the group consisting of an emulsion, lotion, spray, aerosol, powder, ointment, cream and foam.

18. The composition of claim 1, wherein the mammalian tissue being treated is a blood vessel.

19. The composition of claim 1, wherein the composition is useful for treating a condition selected from the group consisting of hypertension, coronary heart disease, arteriosclerosis, angina, coronary thrombosis, chronic obstructive pulmonary disease, and restenosis post angioplasty.

20. A composition useful in improving tissue turgor, said composition being comprised of a peptide selected from the group consisting of Sequence No. 1 (Alanine-Valine-Glycine), Sequence No. 2 (Valine-Glycine-Alanine-Glycine), Sequence No. 3 (Isoleucine-Glycine-Glycine), Sequence No. 4 (Leucine-Glycine), Sequence No. 5 (Isoleucine-Glycine-Alanine-Glycine), Sequence No. 6 (Leucine-Glycine-Glycine), Sequence No. 7 (Valine-Alanine-Proline-Glycine), Sequence No. 8 (Leucine-Glycine-Proline-Glycine), Sequence No. 9 (Leucine-Glycine-Alanine-Glycine), Sequence No. 10 (Valine-Glycine-Proline-Glycine), Sequence No. 11 (Phenylalanine-Glycine-Proline-Glycine), Sequence No. 12 (Valine-Glycine-Proline-Glutamine), Sequence No. 13, (Leucine-Glycine-Alanine), Sequence No. 14 (Valine-Glycine-Proline-Alanine), Sequence No. 15 (Valine-Valine-Proline-Glycine), Sequence No. 16, (Alanine-Valine-Proline-Glycine), Sequence No. 17 (Valine-Valine-Proline-Glutamine), Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine), Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine), Sequence No. 20 (Alanine-Isoleucine-Proline-Glycine), Sequence No. 21 (Leucine-Glycine-Proline-Glycine-Glycine), Sequence No. 22 (Alanine-Alanine-Alanine-Glutamine-Alanine), Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine), Sequence No. 24

(Valine-Tyrosine-Proline-Glycine-Glycine), Sequence No. 25 (Isoleucine-Glycine-Glycine-Valine-Glycine-Glycine), Sequence No. 26 (Valine-Alanine-Proline-Glycine-Valine-Glycine), Sequence No. 27 (Leucine-Glycine-Valine-Glycine-Glycine), Sequence No. 28 (Valine-Leucine-Proline-Glycine), Sequence No. 29 (Phenylalanine-Arginine-Alanine-Alanine-Alanine), Sequence No. 30 (Valine-Glycine-Glycine-Valine-Proline-Glycine), Sequence No. 31 (Phenylalanine-Glycine-Proline-Glycine-Glycine), Sequence No. 32 (Valine-Glycine-Valine-Proline-Glycine), Sequence No. 33 (Valine-Leucine-Proline-Glycine-Alanine-Glycine), Sequence No. 34 (Valine-Glycine-Leucine-Hydroxyproline-Glycine), Sequence No. 35 (Leucine-Glycine-Valine-Glycine-Alanine), Sequence No. 36 (Alanine-Phenylalanine-Proline-Glycine), Sequence No. 37 (Alanine-Phenylalanine-Proline-Glycine-Alanine), Sequence No. 38 (Valine-Glycine-Isoleucine-Proline-Alanine), Sequence No. 39 (Valine-Glycine-Glycine-Isoleucine-Proline-Threonine), Sequence No. 40 (Valine-Glycine-Valine-Glycine-Valine-Proline-Glycine), and Sequence No. 41 (Leucine-Glycine-Proline-Glycine-Valine-Glycine).

21. The composition of claim 20, wherein said composition further includes a pharmaceutical delivery system.

22. The composition of claim 20, wherein said peptide is selected from the group consisting of Sequence No. 14 (Valine-Glycine-Proline-Alanine), Sequence No. 15 (Valine-Valine-Proline-Glycine), Sequence No. 16, (Alanine-Valine-Proline-Glycine), Sequence No. 17 (Valine-Valine-Proline-Glutamine), Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine), Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine), Sequence No. 20 (Alanine-Isoleucine-Proline-

Glycine), Sequence No. 21 (Leucine-Glycine-Proline-Glycine-Glycine), Sequence No. 22 (Alanine-Alanine-Alanine-Glutamine-Alanine), Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine), Sequence No. 24 (Valine-Tyrosine-Proline-Glycine-Glycine), Sequence No. 25 (Isoleucine-Glycine-Glycine-Valine-Glycine-Glycine), Sequence No. 26 (Valine-Alanine-Proline-Glycine-Valine-Glycine), Sequence No. 27 (Leucine-Glycine-Valine-Glycine-Glycine), Sequence No. 28 (Valine-Leucine-Proline-Glycine), Sequence No. 29 (Phenylalanine-Arginine-Alanine-Alanine-Alanine), Sequence No. 30 (Valine-Glycine-Glycine-Valine-Proline-Glycine), and Sequence No. 31 (Phenylalanine-Glycine-Proline-Glycine-Glycine).

23. The composition of claim 20, wherein said peptide is selected from the group consisting of Sequence No. 14 (Valine-Glycine-Proline-Alanine), Sequence No. 15 (Valine-Valine-Proline-Glycine), Sequence No. 16, (Alanine-Valine-Proline-Glycine), Sequence No. 17 (Valine-Valine-Proline-Glutamine), Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine), Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine), Sequence No. 20 (Alanine-Isoleucine-Proline-Glycine), Sequence No. 21 (Leucine-Glycine-Proline-Glycine-Glycine), Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine), and Sequence No. 24 (Valine-Tyrosine-Proline-Glycine-Glycine).

24. The composition of claim 20, wherein said peptide is Sequence No. 17 (Valine-Valine-Proline-Glutamine).

25. The composition of claim 20, wherein said peptide is Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine).

26. The composition of claim 20, wherein said peptide is Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine).

27. The composition of claim 20, wherein said peptide is Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine).

28. The composition of claim 20, wherein said peptide is Sequence No. 32 (Valine-Glycine-Valine-Proline-Glycine).

29. The composition of claim 20, wherein said peptide is derived from elastin.

30. The composition of claim 29, wherein said peptide is derived from animal tissue.

31. The composition of claim 20, wherein said peptide is comprised of a polypeptide having a formula of R_1-Valyl-Valyl-Prolyl-Glutamine-R_2, wherein R_1 is an amino portion of the peptide and R_2 a carboxy portion of the peptide.

32. The composition of claim 21, wherein said pharmaceutical delivery system is selected from the group consisting of a topical delivery system and a subcutaneous delivery system.

33. The composition of claim 32, wherein said topical delivery system is selected from the group consisting of a cosmetic preparation, powder, emulsion, lotion, spray, ointment, aerosol, cream and foam.

34. A method enhancing tissue elasticity, said method being comprised of administering a therapeutically effective concentration of a peptide selected from the group consisting of Sequence No. 1 (Alanine-Valine-Glycine), Sequence No. 2 (Valine-Glycine-Alanine-Glycine), Sequence

No. 3 (Isoleucine-Glycine-Glycine), Sequence No. 4 (Leucine-Glycine), Sequence No. 5 (Isoleucine-Glycine-Alanine-Glycine), Sequence No. 6 (Leucine-Glycine-Glycine), Sequence No. 7 (Valine-Alanine-Proline-Glycine), Sequence No. 8 (Leucine-Glycine-Proline-Glycine), Sequence No. 9 (Leucine-Glycine-Alanine-Glycine), Sequence No. 10 (Valine-Glycine-Proline-Glycine), Sequence No. 11 (Phenylalanine-Glycine-Proline-Glycine), Sequence No. 12 (Valine-Glycine-Proline-Glutamine), Sequence No. 13, (Leucine-Glycine-Alanine), Sequence No. 14 (Valine-Glycine-Proline-Alanine), Sequence No. 15 (Valine-Valine-Proline-Glycine), Sequence No. 16, (Alanine-Valine-Proline-Glycine), Sequence No. 17 (Valine-Valine-Proline-Glutamine), Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine), Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine), Sequence No. 20 (Alanine-Isoleucine-Proline-Glycine), Sequence No. 21 (Leucine-Glycine-Proline-Glycine-Glycine), Sequence No. 22 (Alanine-Alanine-Alanine-Glutamine-Alanine), Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine), Sequence No. 24 (Valine-Tyrosine-Proline-Glycine-Glycine), Sequence No. 25 (Isoleucine-Glycine-Glycine-Valine-Glycine-Glycine), Sequence No. 26 (Valine-Alanine-Proline-Glycine-Valine-Glycine), Sequence No. 27 (Leucine-Glycine-Valine-Glycine-Glycine), Sequence No. 28 (Valine-Leucine-Proline-Glycine), Sequence No. 29 (Phenylalanine-Arginine-Alanine-Alanine-Alanine), Sequence No. 30 (Valine-Glycine-Glycine-Valine-Proline-Glycine), Sequence No. 31 (Phenylalanine-Glycine-Proline-Glycine-Glycine), Sequence No. 32 (Valine-Glycine-Valine-Proline-Glycine), Sequence No. 33 (Valine-Leucine-Proline-Glycine-Alanine-Glycine), Sequence No. 34 (Valine-Glycine-Leucine-Hydroxyproline-Glycine), Sequence No. 35 (Leucine-

Glycine-Valine-Glycine-Alanine), Sequence No. 36 (Alanine-Phenylalanine-Proline-Glycine), Sequence No. 37 (Alanine-Phenylalanine-Proline-Glycine-Alanine), Sequence No. 38 (Valine-Glycine-Isoleucine-Proline-Alanine), Sequence No. 39 (Valine-Glycine-Glycine-Isoleucine-Proline-Threonine), Sequence No. 40 (Valine-Glycine-Valine-Glycine-Valine-Proline-Glycine), and Sequence No. 41 (Leucine-Glycine-Proline-Glycine-Valine-Glycine).

35. The method of claim 34, wherein said peptide is Sequence No. 17 (Valine-Valine-Proline-Glutamine).

36. The method of claim 34, wherein the tissue in which elasticity is enhanced is skin.

37. The method of claim 34, wherein appearance of the skin is enhanced.

38. The method claim 34, further including the step of stimulating endogenous production of elastin.

39. The method of claim 34, wherein the tissue is a blood vessel.

40. The method of claim 34, wherein said tissue is deficient in elastin.

41. The method of claim 34, wherein said tissue is lung tissue.

42. The method of claim 34, wherein the step of administering the peptide is repeated over a predetermined time period.

43. The method of claim 34, wherein the predetermined time period exceeds 14 days of twice daily administration of said peptide.

44. The method of claim 34, wherein said peptide is a hydrophilic elastin peptide.

45. The method of claim 34, wherein said peptide is formed by enzymatic cleavage of elastin with thermolysin.

46. The method of claim 34, wherein said peptide is included in a topical preparation to be applied to a patient's skin.

47. A pharmaceutical composition comprised of a peptide selected from the group consisting of Sequence No. 1 (Alanine-Valine-Glycine), Sequence No. 2 (Valine-Glycine-Alanine-Glycine), Sequence No. 3 (Isoleucine-Glycine-Glycine), Sequence No. 4 (Leucine-Glycine), Sequence No. 5 (Isoleucine-Glycine-Alanine-Glycine), Sequence No. 6 (Leucine-Glycine-Glycine), Sequence No. 7 (Valine-Alanine-Proline-Glycine), Sequence No. 8 (Leucine-Glycine-Proline-Glycine), Sequence No. 9 (Leucine-Glycine-Alanine-Glycine), Sequence No. 10 (Valine-Glycine-Proline-Glycine), Sequence No. 11 (Phenylalanine-Glycine-Proline-Glycine), Sequence No. 12 (Valine-Glycine-Proline-Glutamine), Sequence No. 13, (Leucine-Glycine-Alanine), Sequence No. 14 (Valine-Glycine-Proline-Alanine), Sequence No. 15 (Valine-Valine-Proline-Glycine), Sequence No. 16, (Alanine-Valine-Proline-Glycine), Sequence No. 17 (Valine-Valine-Proline-Glutamine), Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine), Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine), Sequence No. 20 (Alanine-Isoleucine-Proline-Glycine), Sequence No. 21 (Leucine-Glycine-Proline-Glycine-Glycine), Sequence No. 22 (Alanine-Alanine-Alanine-Glutamine-Alanine), Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-

Glycine), Sequence No. 24 (Valine-Tyrosine-Proline-Glycine-Glycine), Sequence No. 25 (Isoleucine-Glycine-Glycine-Valine-Glycine-Glycine), Sequence No. 26 (Valine-Alanine-Proline-Glycine-Valine-Glycine), Sequence No. 27 (Leucine-Glycine-Valine-Glycine-Glycine), Sequence No. 28 (Valine-Leucine-Proline-Glycine), Sequence No. 29 (Phenylalanine-Arginine-Alanine-Alanine-Alanine), Sequence No. 30 (Valine-Glycine-Glycine-Valine-Proline-Glycine), Sequence No. 31 (Phenylalanine-Glycine-Proline-Glycine-Glycine), Sequence No. 32 (Valine-Glycine-Valine-Proline-Glycine), Sequence No. 33 (Valine-Leucine-Proline-Glycine-Alanine-Glycine), Sequence No. 34 (Valine-Glycine-Leucine-Hydroxyproline-Glycine), Sequence No. 35 (Leucine-Glycine-Valine-Glycine-Alanine), Sequence No. 36 (Alanine-Phenylalanine-Proline-Glycine), Sequence No. 37 (Alanine-Phenylalanine-Proline-Glycine-Alanine), Sequence No. 38 (Valine-Glycine-Isoleucine-Proline-Alanine), Sequence No. 39 (Valine-Glycine-Glycine-Isoleucine-Proline-Threonine), Sequence No. 40 (Valine-Glycine-Valine-Glycine-Valine-Proline-Glycine), and Sequence No. 41 (Leucine-Glycine-Proline-Glycine-Valine-Glycine).

48. The pharmaceutical composition of claim 47, wherein said peptide is Sequence No. 17 (Valine-Valine-Proline-Glutamine).

49. The pharmaceutical composition of claim 47, wherein said peptide is Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine).

50. The pharmaceutical composition of claim 47, wherein said peptide is Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine).

51. The pharmaceutical composition of claim 47, wherein said peptide is derived from elastin.

52. The pharmaceutical composition of claim 47, wherein application of said composition to a patient results in neovascularization.

53. The pharmaceutical composition of claim 47, wherein application of said composition to a patient results in angiogenesis.

54. A cosmetic preparation comprised of a peptide, said peptide being selected from the group consisting of Sequence No. 1 (Alanine-Valine-Glycine), Sequence No. 2 (Valine-Glycine-Alanine-Glycine), Sequence No. 3 (Isoleucine-Glycine-Glycine), Sequence No. 4 (Leucine-Glycine), Sequence No. 5 (Isoleucine-Glycine-Alanine-Glycine), Sequence No. 6 (Leucine-Glycine-Glycine), Sequence No. 7 (Valine-Alanine-Proline-Glycine), Sequence No. 8 (Leucine-Glycine-Proline-Glycine), Sequence No. 9 (Leucine-Glycine-Alanine-Glycine), Sequence No. 10 (Valine-Glycine-Proline-Glycine), Sequence No. 11 (Phenylalanine-Glycine-Proline-Glycine), Sequence No. 12 (Valine-Glycine-Proline-Glutamine), Sequence No. 13, (Leucine-Glycine-Alanine), Sequence No. 14 (Valine-Glycine-Proline-Alanine), Sequence No. 15 (Valine-Valine-Proline-Glycine), Sequence No. 16, (Alanine-Valine-Proline-Glycine), Sequence No. 17 (Valine-Valine-Proline-Glutamine), Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine), Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine), Sequence No. 20 (Alanine-Isoleucine-Proline-Glycine), Sequence No. 21 (Leucine-Glycine-Proline-Glycine-Glycine), Sequence No. 22 (Alanine-Alanine-Alanine-Glutamine-Alanine), Sequence No. 23 (Valine-Glycine-Valine-

Hydroxyproline-Glycine), Sequence No. 24 (Valine-Tyrosine-Proline-Glycine-Glycine), Sequence No. 25 (Isoleucine-Glycine-Glycine-Valine-Glycine-Glycine), Sequence No. 26 (Valine-Alanine-Proline-Glycine-Valine-Glycine), Sequence No. 27 (Leucine-Glycine-Valine-Glycine-Glycine), Sequence No. 28 (Valine-Leucine-Proline-Glycine), Sequence No. 29 (Phenylalanine-Arginine-Alanine-Alanine-Alanine), Sequence No. 30 (Valine-Glycine-Glycine-Valine-Proline-Glycine), Sequence No. 31 (Phenylalanine-Glycine-Proline-Glycine-Glycine), Sequence No. 32 (Valine-Glycine-Valine-Proline-Glycine), Sequence No. 33 (Valine-Leucine-Proline-Glycine-Alanine-Glycine), Sequence No. 34 (Valine-Glycine-Leucine-Hydroxyproline-Glycine), Sequence No. 35 (Leucine-Glycine-Valine-Glycine-Alanine), Sequence No. 36 (Alanine-Phenylalanine-Proline-Glycine), Sequence No. 37 (Alanine-Phenylalanine-Proline-Glycine-Alanine), Sequence No. 38 (Valine-Glycine-Isoleucine-Proline-Alanine), Sequence No. 39 (Valine-Glycine-Glycine-Isoleucine-Proline-Threonine), Sequence No. 40 (Valine-Glycine-Valine-Glycine-Valine-Proline-Glycine), and Sequence No. 41 (Leucine-Glycine-Proline-Glycine-Valine-Glycine).

55. The cosmetic preparation of claim 54 wherein said peptide is selected from the group consisting of Sequence No. 14 (Valine-Glycine-Proline-Alanine), Sequence No. 15 (Valine-Valine-Proline-Glycine), Sequence No. 16, (Alanine-Valine-Proline-Glycine), Sequence No. 17 (Valine-Valine-Proline-Glutamine), Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine), Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine), Sequence No. 20 (Alanine-Isoleucine-Proline-Glycine), Sequence No. 21 (Leucine-Glycine-Proline-Glycine-

Glycine), Sequence No. 22 (Alanine-Alanine-Alanine-Glutamine-Alanine), Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine), Sequence No. 24 (Valine-Tyrosine-Proline-Glycine-Glycine), Sequence No. 25 (Isoleucine-Glycine-Glycine-Valine-Glycine-Glycine), Sequence No. 26 (Valine-Alanine-Proline-Glycine-Valine-Glycine), Sequence No. 27 (Leucine-Glycine-Valine-Glycine-Glycine), Sequence No. 28 (Valine-Leucine-Proline-Glycine), Sequence No. 29 (Phenylalanine-Arginine-Alanine-Alanine-Alanine), Sequence No. 30 (Valine-Glycine-Glycine-Valine-Proline-Glycine), and Sequence No. 31 (Phenylalanine-Glycine-Proline-Glycine-Glycine).

56. The cosmetic preparation of claim 54, wherein said peptide is selected from the group consisting of Sequence No. 14 (Valine-Glycine-Proline-Alanine), Sequence No. 15 (Valine-Valine-Proline-Glycine), Sequence No. 16, (Alanine-Valine-Proline-Glycine), Sequence No. 17 (Valine-Valine-Proline-Glutamine), Sequence No. 18 (Valine-Alanine-Alanine-Arginine-Proline-Glycine), Sequence No. 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine), Sequence No. 20 (Alanine-Isoleucine-Proline-Glycine), Sequence No. 21 (Leucine-Glycine-Proline-Glycine-Glycine), Sequence No. 23 (Valine-Glycine-Valine-Hydroxyproline-Glycine), and Sequence No. 24 (Valine-Tyrosine-Proline-Glycine-Glycine).

57. The cosmetic preparation of claim 54, wherein said peptide is Sequence No. 17 (Valine-Valine-Proline-Glutamine).

58. The cosmetic preparation of claim 54, wherein said cosmetic preparation is selected from the group consisting of a powder, emulsion, lotion, spray, ointment, aerosol, cream and foam.

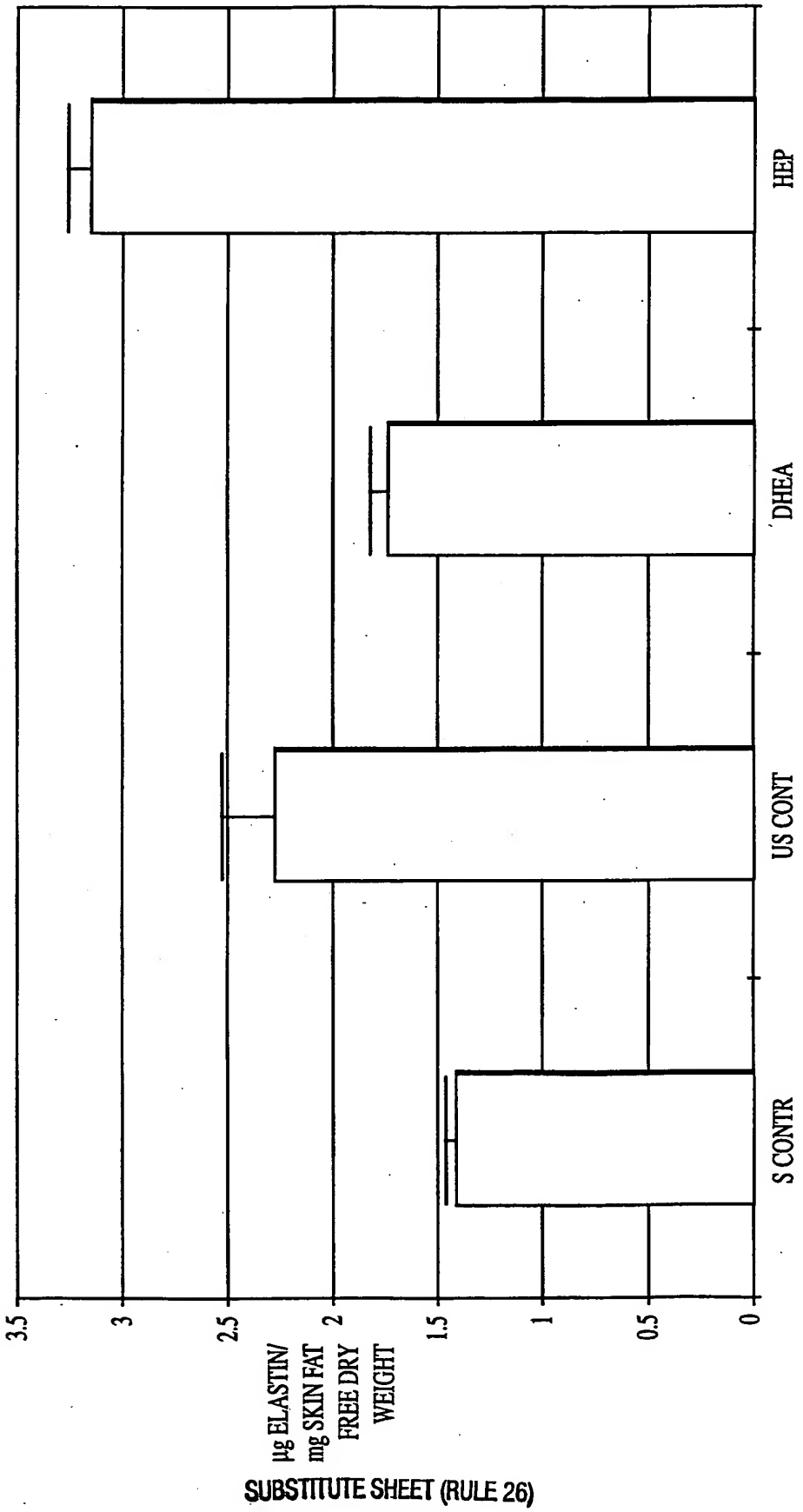
HEP
DHEA
US CONT
S CONTR
3.5
3
2.5
2
1.5
1
0.5
0
µg ELASTIN/
mg SKIN FAT
FREE DRY
WEIGHT

FIG. 1

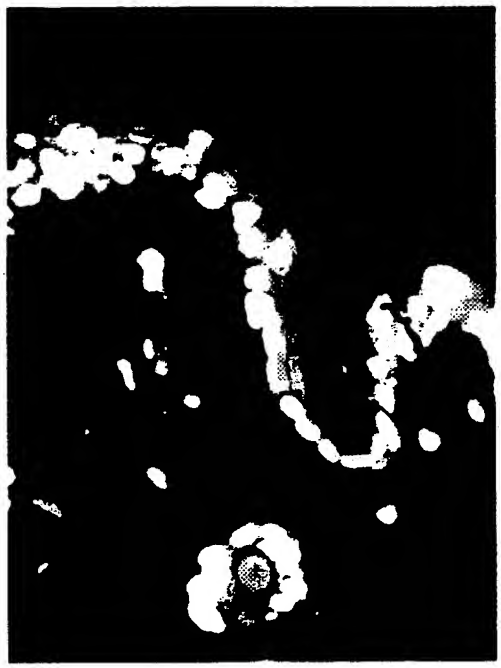

FIG. 2B

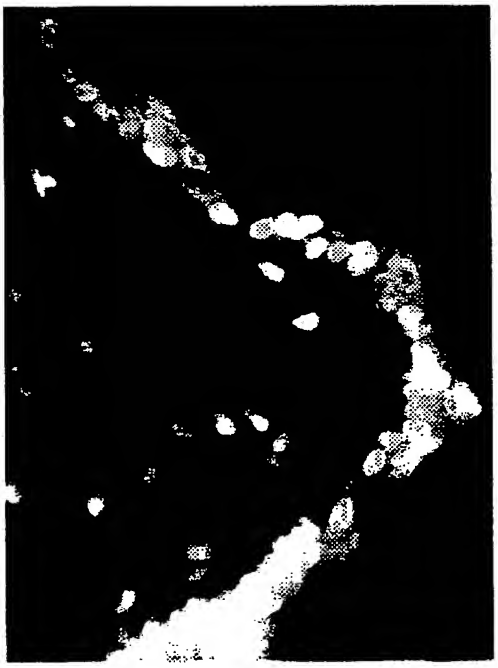

FIG. 2A

FIG. 2D

FIG. 2C

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Sandberg, Lawrence; Roos, Phillip; Mitts, Thomas

(ii) TITLE OF INVENTION: PEPTIDE COMPOSITIONS AND FORMULATIONS AND USE OF SAME

(iii) NUMBER OF SEQUENCES: 41

(iv) CORRESPONDENCE ADDRESS:

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(C) CITY: Pittsburgh
(D) STATE: Pennsylvania
(E) COUNTRY: USA
(F) ZIP: 15230

(v) COMPUTER READABLE FORM

(A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 Mb
(B) COMPUTER: Compaq
(C) OPERATING SYSTEM: Microsoft Windows 95
(D) SOFTWARE: Word 6.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: March 12, 1999
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION
(A) NAME: Miller, Raymond A.
(B) REGISTRATION NUMBER: 42,891
(C) REFERENCE/DOCKET NUMBER: 97-489-WO

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (412) 288-4192
(B) TELEFAX: (412) 288-3300

(2) INFORMATION FOR SEQ ID NO. 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 1:

Ala Val Gly
1

(2) INFORMATION FOR SEQ ID NO. 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO 2:

Val Gly Ala Gly
1

(2) INFORMATION FOR SEQ ID NO. 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO 3:

Ile Gly Gly
1

(2) INFORMATION FOR SEQ ID NO. 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO 4:

Leu Gly
1

(2) INFORMATION FOR SEQ ID NO. 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO 5:

Ile Gly Ala Gly
1

(2) INFORMATION FOR SEQ ID NO. 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 6:

Leu Gly Gly
1

(2) INFORMATION FOR SEQ ID NO. 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 7:

Val Ala Pro Gly
1

(2) INFORMATION FOR SEQ ID NO. 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 8:

Leu Gly Pro Gly
1

(2) INFORMATION FOR SEQ ID NO. 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 9:

Leu Gly Ala Gly
1

(2) INFORMATION FOR SEQ ID NO. 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 10:

Val Gly Pro Gly
1

(2) INFORMATION FOR SEQ ID NO. 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 11:

Phe Gly Pro Gly
1

(2) INFORMATION FOR SEQ ID NO. 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 12:

Val Gly Pro Gln
1

(2) INFORMATION FOR SEQ ID NO. 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 13:

Leu Gly Ala
1

(2) INFORMATION FOR SEQ ID NO. 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 14:

Val Gly Pro Ala
1

(2) INFORMATION FOR SEQ ID NO. 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 15

Val Val Pro Gly
1

(2) INFORMATION FOR SEQ ID NO. 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 16:

Ala Val Pro Gly
1

(2) INFORMATION FOR SEQ ID NO. 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 17:

Val Val Pro Gln
1

(2) INFORMATION FOR SEQ ID NO. 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 18:

Val Ala Ala Arg Pro Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 19:

Leu Gly Ala Gly Gly Ala Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 20:

Ala Ile Pro Gly
1

(2) INFORMATION FOR SEQ ID NO. 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 21:

Leu Gly Pro Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 22:

Ala Ala Ala Gln Ala
1 5

(2) INFORMATION FOR SEQ ID NO. 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 23:

Val Gly Val Xaa Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 24:

Val Tyr Pro Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 25:

Ile Gly Gly Val Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 26:

Val Ala Pro Gly Val Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 27:

Leu Gly Val Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 28:

Val Leu Pro Gly
1

(2) INFORMATION FOR SEQ ID NO. 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 29:

Phe Arg Ala Ala Ala
1 5

(2) INFORMATION FOR SEQ ID NO. 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 30:

Val Gly Gly Val Pro Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 31:

Phe Gly Pro Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 32:

Val Gly Val Pro Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 33:

Val Leu Pro Gly Ala Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 34:

Val Gly Leu Xaa Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 35:

Leu Gly Val Gly Ala
1 5

(2) INFORMATION FOR SEQ ID NO. 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 36:

Ala Phe Pro Gly
1

(2) INFORMATION FOR SEQ ID NO. 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 37:

Ala Phe Pro Gly Ala
1 5

(2) INFORMATION FOR SEQ ID NO. 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 38:

Val Gly Ile Pro Ala
1 5

(2) INFORMATION FOR SEQ ID NO. 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 39:

Val Gly Gly Ile Pro Thr
1 5

(2) INFORMATION FOR SEQ ID NO. 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 40:

Val Gly Val Gly Val Pro Gly
1 5

(2) INFORMATION FOR SEQ ID NO. 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO 41:

Leu Gly Pro Gly Val Gly
1 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/05496

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00, 38/06, 38/07, 38/08
US CL : 514/02,16,17,18; 530/300,329, 330, 331

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/02,16,17,18; 530/300,329, 330, 331

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN ON LINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database CAPLUS, AN 115:65185, DOI, R. et al., Effects of synthetic human pancreastatin on pancreatic secretion and blood flow in rats and dogs. Peptides. 1991, Vol. 12(3), pages 499-502.	31
X	Database CAPLUS, AN 107:54378, RAJU, K. et al., Primary structure of bovine elastin a, b, and c deduced from the sequences of cDNA clones. J. Biol. Chem., 1987, 262(12), pages 5755-5762.	31
X - Y	JP 08225594 A2 (YAMAUCHI ET AL) 09 March 1993, see entire document.	14, 18-21, 29 30,47,51-54 ---------- 12-17,32,33,58

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 JUNE 1999	Date of mailing of the international search report 24 JUN 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL BORIN for D. Lawrence Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/05496

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	Database CAPLUS, AN 107:191131, CHARTEN et al., QSAR for peptide bioactivities. Further studies. Pharmacochem. Libr. 1987, Vol. 10, pages 285-290.	1-3,5,6,18,19,22, 23,29,30, 47,51-56 --------- 12-17, 32,33,58
X - Y	Database CAPLUS, 129:187343, LOGRANO, M. et al., Identification of elastin peptides with vasorelaxant activity on rat thoracic aorta. Int. J. Biochem. Cell Biol., 1998, Vol. 30, pages 497-503.	13, 10,18, 19,28,29,30 51-53 -------- 12-17, 32,33,58
A	HUNNINGHAKE, G.W. et al. Elastin fragments attract macrophage precursors to diseased sites in pulmonary emphisema. Science. 1981, Vol. 212, pages 925-927.	1-33, 47-58

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/05496

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-33, 47-58

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.